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BIOCHEMICAL STUDIES ON THE RICE BLAST DISEASE (PART 10)
BIOCHEMICAL CLASSIFICATION OF PIRICULARIA ORYZAE (NO 3)

- Japan -

[Following is a translation of an article by Otsuka, H., et al., in the Japanese-language Agricultural Chem. Soc. Japan Journal, Vol 31, 1957, pages 886-890.]

Attempts have been made to classify Piricularia oryzae Cavara in terms of bacterial reactions to several varieties of rice (1, 2), and we have noticed a close relationship between them and the method in our report Part 10 (No 2), which depended on the extent of the sugar consumption of 45 stock-cultures of Piricularia oryzae Cavara (3).

Recently Burkholder and others (4) have demonstrated the possibility of classifying ray bacteria by the differential amount of use by them of nitrogen sources, and this is of great interest with respect to the problem of bacteria classification.

Otani (5) studied the degree of consumption of nitrogen sources by Piricularia oryzae Cavara and showed that nitric acid-derived nitrogen, glycocoll, L-alaninine, aspartic acid, asparagine, and D-glutamic acid constitute excellent nitrogen sources, while DL-α-amino butyric acid, creatine, and taurine are not adequate as nitrogen sources.

Tanaka and Kogetsu (6), on the other hand, studied the effects of the ammonia family and of nitric acid-derived nitrogen on nutrition absorption in Piricularia oryzae Cavara (hereafter called P.o.c.) and demonstrated that the prevention of Ph acidification of the culture medium results in effective use and assimilation of ammonia

In addition, there is a study by Leaver (7) that indicates the effective use of organic nitrogen, particularly amino acid, by P.o.c.

Using the 45 P.o.c. stock-cultures as described in our report Part 10 (No 1) (8), we have investigated the degree of consumption of various inorganic as well as organic nitrogens, amino acid in particular, and noted a great amount of stock-culture difference among them. The present study reports on such stock-culture differences, were based on the classification table in our report Part 10 (No 2).

Experiment

1) P.o.c. used in the experiment: the 45 stock-cultures which were used in report Part 10 (No 1)

2) Pre-cultivation: as described in the report Part 10 (No 1)

3) Culture medium for the use of nitrogen-source: The composition of the culture medium is as shown in Table 1.

Table 1

Composition of the culture medium for the use of nitrogen source

1) グルコース	20 g
Nitrogen-Source	0.4154 g
	(KNO_3 3 g に相当) 5)
K_2HPO_4	1.0 g
KH_2PO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
$CaCl_2$	0.1 g
$FeSO_4 \cdot 7H_2O$	0.0075 g
$MnSO_4 \cdot 7H_2O$	0.002 g
$CuSO_4 \cdot 5H_2O$	0.006 g
$ZnCl_2$	0.075 g
$(NH_4)_6MO_7O_{24} \cdot 4H_2O$	0.009 g
2) ビオチン	5 γ
3) チアミン	1 mg
4) 蒸留水を加え 1l とす (pH 7.0)	

[Legend:] 1) Glucose; 2) Biotine; 3) Thiamine; 4) Make it 1l by adding distilled water; 5) equivalent to.

The solution of the compound without the glucose, nitrogen source, biotine and vitamine B_1 was sterilized for 15 min.

at 15 pounds pressure, and then glucose, the nitrogen source, biotine and vitamine B_1 were added. After dissolving the resulting compound, 2 ml. each was transferred into test tubes of 17 mm diameter and further sterilized for 10 min. at 10 lb. before being put to use as the culture medium.

4) Determination of the extent of consumption: Each stock-culture was cultivated in the culture medium for 14 days at 25°, and was washed with water and dried at 100° to a constant quantity (4-6 hr.). The extent of nitrogen consumption was determined from the relative weight of the dried bacteria. The results are shown in Table 2.

Table 2

Results on the extent of nitrogen source consumption (weight of bacteria gx200)

菌株 / 窒素源	5414	5418	3	No. 1	No. 2	No. 1. F8[illegible]	No. 11[illegible]	No. 188[illegible]	F[illegible]	A 25	A 36	5309	5311	5327	5330
対照*	0.05	0.10	0.05	0.05	0.05	0.0[illegible]	0.05	0.10	[illegible]	0.05	0.05	0.10	0.05	0.20	0.10
アスパラギン	2.80	1.85	2.60	2.90	0.10	1.7[illegible]	3.30	2.70	[illegible]	1.80	3.35	3.20	2.95	3.10	3.30
KNO_3	1.35	3.25	2.80	2.10	3.60	2.2[illegible]	3.10	2.50	[illegible]	3.50	0.70	3.10	3.30	3.70	2.40
$NaNO_3$	1.15	3.40	3.10	3.50	3.60	3.6[illegible]	3.65	2.60	[illegible]	2.75	2.60	3.40	3.60	1.90	2.80
$NaNO_3$	1.00	1.25	1.15	2.10	2.80	0.0[illegible]	0.50	0.20	[illegible]	1.80	0.25	2.20	2.00	2.80	2.30
トレオニン	2.80	4.35	2.80	3.20	3.25	3.8[illegible]	5.20	4.15	[illegible]	3.70	2.50	0.80	3.35	2.90	2.55
アスパラギン酸	3.50	3.15	2.80	3.90	4.90	3.7[illegible]	[illegible]	1.65	[illegible]	3.60	2.40	3.50	3.10	3.20	3.80
アルギニン	3.05	3.80	2.60	3.60	3.90	2.7[illegible]	3.50	1.85	[illegible]	3.60	2.40	3.20	3.90	3.00	2.90
チロシン	1.20	1.20	2.00	2.20	2.10	2.5[illegible]	1.70	1.50	[illegible]	1.50	1.40	1.75	1.90	2.10	1.80
アラニン	2.10	4.35	2.70	4.00	4.40	4.1[illegible]	3.70	3.13	[illegible]	2.50	3.80	3.50	2.50	4.05	2.10
フェニルアラニン	0.50	0.70	1.00	0.80	1.40	1.3[illegible]	0.20	0.25	[illegible]	1.20	0.50	1.00	5.10	0.60	0.50
プロリン	3.20	3.00	3.05	3.85	4.40	2.8[illegible]	3.80	3.50	[illegible]	3.70	3.60	3.40		3.60	3.20
シスチン	0.65	0.10	1.10	1.30	1.50	1.0[illegible]	[illegible]	0.25	[illegible]	1.20	0.00	0.40	1.30	0.40	0.05
Hydroxy proline	0.10	0.10	0.20	0.20	0.30	0.1[illegible]	0.10	0.10	[illegible]	0.05	0.00	0.05	0.50	0.10	0.20
ロイシン	2.70	3.10	2.75	1.60	1.80	3.2[illegible]	2.90	1.20	[illegible]	2.30	0.60	2.10	2.30	1.20	2.95
メチオニン	2.00	1.70	1.15	2.40	2.60	2.7[illegible]	2.00	1.95	2.00	1.70	1.30	2.80	2.55	2.10	0.50
イソロイシン	2.30	3.10	3.60	3.10	2.70	3.2[illegible]	2.55	1.60	2.80	2.60	2.00	2.00	3.00		3.80
セリン	2.50	2.10	3.55	3.85	3.70	3.6[illegible]	3.10	3.40	[illegible]	3.30	2.90	3.30	2.60	3.45	3.60
グリシン	3.07	3.07	2.85	4.40	4.20	4.0[illegible]	3.85	3.20	[illegible]	4.10	2.20	3.65	3.50	3.10	4.10
バリン	2.20	2.07	2.80	2.30	2.95	3.3[illegible]	4.00	1.50	[illegible]	3.80	2.00	2.80	3.20	3.40	2.30
ヒスチジン	3.80	3.00	3.10	2.20	3.60	3.8[illegible]	3.80	3.40	[illegible]	3.90	2.30	2.75	3.60	3.50	3.10
グルタミン酸	2.40	3.20	3.85	4.40	4.50	4.8[illegible]	4.00	3.50	[illegible]	3.40	4.10	4.10	2.70	3.40	3.60
リジン	1.35	1.05	2.40	2.50	2.20	2.1[illegible]	3.00	0.80	[illegible]	2.30	0.25	1.80	2.60		1.40
トリプトファン	0.80	1.00	1.80	1.50	3.00	2.7[illegible]	[illegible]	0.25	[illegible]	1.80	0.10	0.50	1.00	2.80	1.40

[Table on following page]

窒素源 \ 菌株	5333	5404	5420	5424	5425	[illegible]	[illegible]	5515	[illegible]	5517	5518	5519	5520	5521	5522
対照*	0.30	0.05	0.30	0.20	0.05	0.[illegible]	0.05	0.20	[illegible]	0.05	0.20	0.00	0.10	0.10	0.05
アスパラギン	3.40	3.50	2.50	3.20	3.35	3.[illegible]	3.20	3.50	3.2[illegible]	3.50		3.40	2.75	3.30	3.00
KNO_3	1.00	2.05	0.70	2.20	3.05	2.[illegible]	2.30	3.70	1.60	3.20		2.80	2.80	2.50	1.60
$NaNO_2$	1.40	3.50	0.60	2.30	3.80	2.[illegible]	3.20	3.60	1.90			3.00	2.20	3.40	3.00
$NaNO_3$	2.70	1.00	0.30	1.20	0.50	3.[illegible]	0.30	2.10	1.2[illegible]	0.40		2.70	1.10	1.20	0.10
トレオニン	3.35	2.10	3.60	1.70	2.95	[illegible]	2.[illegible]0	1.80	[illegible]	1.20	1.20	2.35	1.90	3.00	3.65
アスパラギン酸	3.20	3.60	3.20	3.20	3.00	3.[illegible]	3.80	3.00	[illegible]	3.90		3.00	3.00	3.60	3.70
アルギニン	2.60	3.30	3.10	3.90	3.70	3.[illegible]0	3.30	2.55	[illegible]	2.80		3.20	2.00	3.70	2.50
チロシン	0.85	2.40	1,25	0.70	1.25	1.[illegible]0	1.70	2.20	[illegible]	1.40	1.75	0.90	1.70	1.50	1.20
アラニン	2.60	2.80	3.60	3.60	3.60	3.[illegible]5	3.30	4.10	[illegible]	3.20	2.15	2.30	2.10	2.70	3.45
フェニルアラニン	0.70	1.05	2.90	1.50	1.20	0.[illegible]	0.00	1.00	[illegible]	0.80		1.00	0.80	0.70	0.70
プロリン	3.30	3.40	2.40	3.20	3.40	3.[illegible]	3.60	2.70	3.1[illegible]	2.80		3.15	3.10	2.90	2.80
シスチン	0.80	1.05	0.50	0.70	0.40	0.[illegible]5	0.[illegible]0	0.60	1.2[illegible]	0.50		1.30	0.30	0.70	0.70
Hydroxy proline	0.05	0.10	0.30	0.10	0.20	0.[illegible]0	0.10	0.10	0.10	0.20		0.20	1.10	0.30	0.10
ロイシン	1.90	1.10	2.00	1.30	1.20	1.[illegible]	1.80	2.00	2.[illegible]	1.40		2.20	2.45	1.20	0.60
メチオニン	2.45	2.90	1.95	0.80	2.50	1.[illegible]0	1.50	2.80	1.80	2.40	0.75	1.30	0.65	1.40	2.00
イソロイシン	3.20	1.90	1.70	3.00	3.00	3.[illegible]0	2.00		2.50			3.70	1.50		
セリン	3.40	2.80	3.35	2.40	3.60	3.[illegible]0	3.50	3.60	3.[illegible]0	4.05	2.80	2.85	3.50	3.70	3.30
グリシン	3.85	3.00	2.70	3.50	2.50	3.70	3.50	3.50	3.60	3.50		3.30	3.25	3.60	3.30
バリン	2.40	2.00	0.80	2.00	2.30	2.[illegible]0	1.80	3.50	1.60	2.00		3.10	1.30	3.70	1.10
ヒスチジン	2.05	4.20	1.40	2.50	4.20	3.[illegible]0	1.40	4.20	2.70	3.10		3.00	4.30	1.80	1.80
グルタミン酸	2.80	3.50	3.25	2.90	3.65	3.[illegible]0	3.60	3.75	3.30	3.10	3.00	2.70	3.80	3.90	3.55
リジン	1.60	2.80	1.00	1.00	1.30	1.[illegible]0	2.00	1.20	1.80	1.40		2.50	1.10	1.90	1.80
トリプトファン	1.40	3.50	0.50	0.80	0.40	0.[illegible]0	0.30	3.70	0.60	2.40		1.70	0.80	1.75	0.80

窒素源 \ 菌株	5523	5524	5525	5526	5527	5528	5529	5532	5533	5534	5535	5536	5537	5539	5540
対照*	0.20	0.05	0.05	0.05	0.05	0.05	0.[illegible]5	0.10	0.20	0.20	0.20	0.05	0.30	0.05	0.10
アスパラギン	3.40	1.70	3.45	3.80	3.30	3.75	3.[illegible]0	3.10	3.40	3.15	3.20	3.50	3.30	3.00	2.20
KNO_3	3.50	0.70	3.00	3.40	1.15	2.50	3.[illegible]	3.00	3.20	3.40	3.60	3.70	3.50	1.85	3.90
$NaNO_3$	3.40	1.85	1.50	3.80	3.00	3.40	3.[illegible]0	3.40	3.20	3.30	4.00	3.50	3.40	3.20	2.90
$NaNO_3$	1.00	0.03	0.65	0.05	0.75	1.80	2.[illegible]0	0.80	0.80	1.60	3.10	2.60	2.20	0.30	1.90
トレオニン	3.10	3.90	3.20	3.90	3.20	3.00	1.[illegible]0	2.25	3.70	3.[illegible]0	3.00	3.20	4.50	1.40	0.70
アスパラギン酸	3.50	3.40	3.30	3.40	3.30	4.20	3.[illegible]0	3.70	3.30	3.50	3.75	3.20	3.20	3.10	3.20
アルギニン	3.30	3.00	3.50	3.40	3.30	3.80	3.[illegible]	3.40	3.15	3.6[illegible]	2.60	3.70	3.50	3.40	2.30
チロシン	1.00	0.80	1.80	1.00	1.10	1.70	1.[illegible]	1.20	1.50	1.60	1.25	1.00	1.15	1.15	1.65
アラニン	3.85	2.90	3.15	3.70	3.45	3.90	4.[illegible]	3.85	2.50	3.[illegible]0	3.40	3.45	2.20	3.20	2.00
フェニルアラニン	1.00	0.00	0.60	0.80	3.20	0.60	0.[illegible]	1.40	1.20	2.50	1.50	1.40	1.20	0.05	0.60
プロリン	2.70	2.35	3.90	3.25	3.50	3.60	3.[illegible]	3.90	2.70	3.[illegible]0	3.55	3.15	3.20	2.90	2.80
シスチン	1.00	0.00	0.40	0.40	0.40	1.15	0.7[illegible]	1.20	1.40	0.60	1.20	1.20	1.25	0.30	0.20
Hydroxy proline	0.15	0.10	0.10	0.10	0.20	0.20	0.2[illegible]	0.10	0.10	0.30	0.15	0.10	0.10	0.20	0.05
ロイシン	1.70	1.20	1.80	1.35	1.60	2.05	2.[illegible]	1.50	1.10	3.[illegible]0	2.10	2.00	3.40	1.60	0.90
メチオニン	1.00	1.50	2.20	1.90	1.80	2.40	2.9[illegible]	1.50	2.00	2.[illegible]0	2.00	0.90	3.60	2.40	0.75
イソロイシン	3.70	2.20	2.45	3.20	2.90	2.40	3.5[illegible]	3.00	2.70	3.[illegible]		2.80	3.60	1.50	
セリン	3.30	3.25	3.60	3.50	3.60	3.15	3.6[illegible]	3.20	3.60	3.[illegible]0	3.50	2.95	2.50	3.00	2.50
グリシン	3.40	3.40	3.30	3.50	3.50	4.00	4.4[illegible]	3.70	2.90	3.[illegible]0	3.70	3.70	3.90	3.40	1.65
バリン	2.70	1.35	1.90	1.00	1.40	2.60	2.5[illegible]	2.50	3.50	2.70	2.75	3.20	3.40	1.80	1.60
ヒスチジン	4.00	1.70	2.90	1.05	1.40	4.30	3.7[illegible]	3.45	3.10	3.60	3.50	4.60	2.25	4.00	1.30
グルタミン酸	3.50	3.30	3.70	3.80	3.30	3.50	4.0[illegible]	3.20	3.50	3.30	3.05	3.50	2.55	2.70	2.70
リジン	1.75	1.80	1.10	1.10	1.00	1.80	1.0[illegible]	1.30	2.30	2.10	1.20	2.20	2.00	1.40	1.90
トリプトファン	0.90	1.00	0.40	0.90	0.30	0.60	0.7[illegible]	0.60	1.50	2.[illegible]0	2.00	2.30	1.80	0.70	1.00

* 対照とは第1表に示した培地組成より窒素源をのぞいた培地での菌体[illegible]す.

[Legend on following page]

[Legend:] 1) Stock culture; 2) Nitrogen source; 3) Control; 4) Asparagine; 5) Threonine; 6) Aspartic acid; 7) Arginine; 8) Tyrosine; 9) Alanine; 10) Phenyl-alanine; 11) proline; 12) Cystine; 13) Leucine; 14) Methionine; 15) Isoleucine; 16) Serine; 17) Glycine; 18) Barine; 19) Histidine; 20) Glutamic acid; 21) Lysine; 22) Tryptophane; 23) By control is meant the bacteria weight in the culture medium of Table 1 from which all the nitrogen sources are removed.

5) Nitric acid reducibility: The culture medium was composed of Zapeck solution (glucose 3%) and 0.2% polypeptone. 7 and 14 days after the inoculation, ordinary tests were carried out on the cultivation filtered solution, the results of which are given in Table 3.

Table 3

Nitric acid reducibility of P.o.c.

① 培養日数 / ③ 菌株	7 ② 日後	14 日後	① 培養日数 / ③ 菌株	7 ② 日後	14 日後	① 培養日数 / ③ 菌株	7 ② 日後	14 日後
5414	+	++	5333	+	−	5523	+	+
5418	+	±	5404	+	+	5524	+	+
3	++	±	5420	+	+	5525	+	−
No. 1	+	±	5424	+	+	5526	+	+
No. 2	+	+	5425	+	+	5527	+	+
No. 11 F 8 hetero	−	−	5415	+	±	5528	+	+
No. 11 hetero	+	±	5514	+	+	5529	±	−
No. 188 hetero	±	−	5515	+	−	5532	+	−
P_3	+	++	5516	+	−	5533	+	+
A 25	+	+	5517	++	−	5534	+	[illegible]
A 36	±	±	5518	+	+	5535	+	
5309	+	−	5519	+	−	5536	+	−
5311	+	±	5520	+	+	5537	+	[illegible]
5327	+	±	5521	+	++	5539	−	[illegible]
5330	+	+	5522	+	+	5540	+	[illegible]

④ 備考：++ 硝酸還元作用強し, + 硝酸還元作用あり, ± 硝酸還元作用疑わし, − [illegible]

[Legend:] 1) The number of days of cultivation; 2) days after (e.g. 7 days after inoculation, etc.); 3) Stock-culture; 4) Comments; ++ -- Presence of strong nitric acid reducibility; + -- Presence of nitric acid reducibility; ± -- Doubtful presence of acid reducibility; − -- Absence of nitric acid reducibility.

Discussion

Excellent nitrogen sources for the growth of P.o.c. in general are asparagine, aspartic acid, arginine, alanine, proline, serine, glycine, histidine, glutamic acid and $NaNO_3$, while such substances as barine, threonine, iso-leucine and NO_2 constitute a fair source of nitrogen. On the other hand, the following substances do not provide a good source of nitrogen; oxiproline, cystine, phenylalanine, tryptophane and $NaNO_2$.

Furthermore, some stock-cultures propagate well on phenyl-alanine, cystine, tryptophane and $NaNO_2$, while other stock-cultures do not propagate at all.

Having been converted from NO_3 by P.o.c., NO_2 could be used as nitrogen source. For this reason the determination of nitric acid reducibility was carried out 7 days or 14 days after the inoculation. Considering both the nitric acid reducibility and the extent of consumption with $NaNO_2$ as nitrogen source (Table 3), we conclude that the following four stock-culture do not show any sign of nitric acid reducibility: No 11 F8 hetero, No 188 hetero, A36 and 5539.

Among the stock-cultures that show nitric acid reducibility, one can distinguish those that utilize $NaNO_2$ from the rest that do not utilize it.

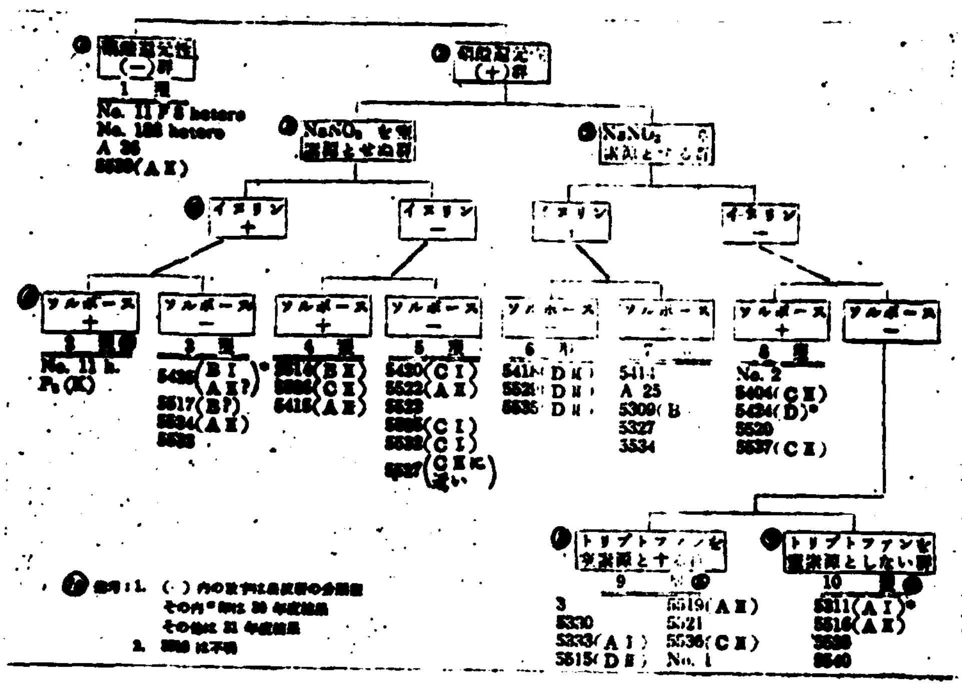
We believe that nitric acid reducibility and the use of $NaNO_2$ will provide important clues in classifying the P.o.c. Therefore, we have revised Table 3 of our report Part 10 (No 2) in terms of nitric acid reducibility and the use of $NaNO_2$ and tryptophane. The revision is shown in Table 4.

The present classification is much simpler than that in our report Part 10 (No 2). Firstly, much is simplified in the matter of poorly propagating organic salts. Secondly the inulin-, sorbose- and glycerol(+)-based typology was previously further subdivided in terms of the Na-citrate, mannite and Na-succinate, whereas in our present scheme the use of $NaNO_2$ and, further, the use of tryptophane provide the classificatory criteria.

Type 1 in our report Part 10 (No 2) included both type K and type D 11 of Agr. Res. Ctr. In contrast to this, however, type 2 of the present classification includes type K only, and D 11 is included in type 7.

Table 4

Classification of Piricularia oryzae Cavara



[Legend:] 1) Negative nitric-acid reducibility; 2) Positive nitric-acid reducibility; 3) Those not using $NaNO_2$ as nitrogen source; 4) Inulin; 5) Sorbose; 6) type; 7) Those using $NaNO_2$ as nitrogen source; 8) Those using tryptophane as nitrogen source; 9) Those not using tryptophane as nitrogen; 10) Remarks -- (1) The number inside ( ) is the classification type of the Agricultural Res. Ctr. The ones with asterik are the results obtained (i.e., 1955.); (2) 5518 is doubtful.

Similarly, A 1, A 11, C 1 and C 11 of Agr. Res. Ctr. were all found in type 6 of our previous scheme, whereas only A 1 and A 11 are included, as shown, in type 9 and 10.

Thus, we note that the etiologically based classification of Agr. Res. Ctr. is somowhat similar to our biochemically based classification, suggesting the possibility that

etiological and biochemical characteristics are rather alike to a large extent.

We do not believe that a calssification scheme based on nitrogen- and carbon- sources exhausts the possibilities for classification. We feel that with increased information on the biochemical properties a better classification system would become feasible.

Summary

Experimenting with the 45 stock-cultures of P.o.c. on the extent of their consumption of various nitrogen sources, amino acid in particular, ~~we have obtained~~ the following results;

1. The following substances are excellent nitrogen-sources -- asparagine, aspartic acid, arginine, alanine, proline, serine, glycine, histidine, glutamic acid and $NaNO_3$, while such substances as oxiproline, cystine, phenylalanine, tryptophane and $NaNO_2$ provide a poor source.

2. The four stock-cultures No 11 F8 hetero, No 188 hetero, A 36 and 5539 do not show nitric acid reducibility, while the remaining 41 stocks show the sign of nitric acid reducibility.

3. The 45 stock-cultures of P.o.c. were classified into 10 families in terms of nitric acid reducibility and the use of $NaNO_2$ and tryptophane in its propagation.

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